151* Biofilm formation and antimicrobial susceptibility of *P. aeruginosa* isolates cultured before and after antibiotic treatment of an acute exacerbation of pulmonary infection

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Introduction and Aims: Exposure of bacteria such as *P. aeruginosa*, growing within a biofilm in the lungs of CF patients, to antibiotics during treatment of recurring pulmonary exacerbations, may result in the development of antibiotic resistance. The aim of this study was to compare biofilm formation and antibiotic susceptibility of matched *P. aeruginosa* isolates cultured from CF sputum before and after antibiotic treatment of an acute exacerbation of pulmonary infection.
Methods: Biofilm formation (24 hours) by 10 matched pairs of *P. aeruginosa* isolates, cultured from sputum samples prior to commencing and at the end of antibiotic treatment, was assessed by total viable count using the Calgary Biofilm Device. The susceptibility of these isolates to antibiotics used in the treatment of CF pulmonary infection (ceftazidime [CAZ], tobramycin [TOB], piperacillin/tazobactam [PIP/TAZ] and meropenem [MER]) was determined using E-test® strips.
Results: All isolates formed biofilms with no differences in biofilm formation apparent between any of the matched pairs of isolates. Prior to commencing antibiotic treatment, *P. aeruginosa* isolates from 8 (CAZ), 10 (TOB), 8 (PIP/TAZ) and 6 (MER) patients were susceptible. Following antibiotic treatment, the susceptibility status of isolates changed from sensitive to resistant for 3 (CAZ), 2 (TOB), 1 (PIP/TAZ) and 3 (MER) patients.
Conclusion: These results indicate that antibiotic treatment had no effect on the ability of *P. aeruginosa* isolates to form bacterial biofilms but in some patients resulted in the development of antibiotic resistance.

152 Correlation of biofilm formation ability and genomic relatedness in *Pseudomonas aeruginosa* strains isolated from cystic fibrosis patient sputa

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Chronic lung infections with *Pseudomonas aeruginosa* have been considered to be the main cause of death for Cystic Fibrosis (CF) patients. In these infections, these and other bacterial species may be growing as biofilms that are more resistant to antibiotic challenge than planktonic cells. In this study, thirty five *P. aeruginosa* strains isolated from sputum sampled from CF patients were assessed for their ability to form biofilms *in vitro*. The degree to which these strains were able to form biofilms was found to differ markedly. This finding led to the hypothesis that this phenotypic differentiation may reflect a difference at the genomic level between strains of *P. aeruginosa*. To test this, Enterobacterial Repetitive Intergenic Consensus (ERIC)-PCR and RAPD-PCR was used to assess the degree of genomic similarity between the strains. These molecular profiling data as assessed by cluster analysis separated the thirty five strains into four distinct groups (A to D). Of these, twelve strains most able to form biofilms were found in groups C and D. This suggested a significant connection between the biofilm development and the genomic relatedness in the strains of *P. aeruginosa*. The significance of these findings is discussed.
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153 Mixed species biofilm formation by aerobic and anaerobic bacteria isolated from the sputum of patients with cystic fibrosis

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Introduction and Aims: We have shown previously by culture that the lungs of Cystic Fibrosis (CF) patients are not only chronically infected with known pathogens, such as *Pseudomonas aeruginosa*, but also by an array of other bacterial species, many of which are anaerobes. As *P. aeruginosa* grows within a biofilm in the lungs of CF patients and as biofilm formation is considered an important virulence factor for CF pulmonary infection, the aim of this study was to determine the ability of anaerobic bacteria to form single and mixed species biofilms with *P. aeruginosa*.
Methods: Biofilm formation (24 & 48 hours) by *Prevotella melaninogenica* and *P. aeruginosa* isolates which had been cultured from the same sputum sample was assessed by total viable count using a micro-titre tray assay. Adherent biofilms were removed from the wells of the micro-titre tray by scraping and the total viable count of both isolates determined by serial dilution and plating on selective agars.
Results: Biofilm formation by both isolates was similar when they were grown alone and in combination for 24 h with the number of adherent bacteria exceeding 10^7 CFU/well. Furthermore, establishment of a *P. aeruginosa* biofilm (24 h) did not prevent the formation of a mixed *P. aeruginosa/Pr. melaninogenica* biofilm (48 h) when *Pr. melaninogenica* was added after 24 h and vice-versa.
Conclusion: These results indicate that *P. aeruginosa* and *Pr. melaninogenica* can form mixed species biofilms in vitro when added both simultaneously and sequentially 24 hours apart.
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154* Origin of lactate in CF sputum

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In CF sputum, lactate can be found in high concentrations. It may be derived from bacterial or cellular sources. Here we correlated lactate in sputum of 25 CF patients to their respective lung functions. In addition, we measured lactate in neutrophils from healthy donors, and in bacterial suspensions of *P. aeruginosa*, *S. aureus*, and *B. cenocepacia* grown aerobically and anaerobically. Anaerobic gene expression of *P. aeruginosa* strain PAO1 was determined using Affymetrix® microarrays. Lactate concentrations in CF sputum came to 3.0±3.1 mmol/L (range 0.2 to 14.1 mmol/L). Neither *P. aeruginosa*, nor *B. cenocepacia* produced lactate in vitro, whereas *S. aureus* generated small amounts. The regulation for *P. aeruginosa* lactate dehydrogenase genes was not affected significantly after one day of anaerobic growth (*ldhA*: –3.9-fold, *lldA*: –1.5-fold, *lldD*: –2.7-fold). The main lactate producers were anaerobically grown neutrophils (3.2 mmol/L). The correlation coefficient between lactate in sputum and FEV1 came to $r = -0.431$ for all patients ($r = -0.527$ for patients harbouring *P. aeruginosa*, and $r = -0.454$ for *S. aureus* patients). Lactate predominantly stems from neutrophils metabolizing anaerobically. Anaerobic *P. aeruginosa* gene regulation corresponds to the missing lactate production. Lactate may be used as a marker of inflammation in CF sputum.
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